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Modulation of Ion Channels: A "Current" View of AKAPs

Minireview

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Deciphering the molecular organization of synapses and the neuronal signaling events at the postsynaptic density is shedding light on the complexity of ion channel modulation. Neuronal activity is mediated by changes in electrical activity, which depends on the movement of ions across the cell membrane through ion channels. Thus, modulating the activity of ligand- and voltage-gated ion channels allows the neuron to modify its response to a variety of stimuli. Defining the molecular mechanisms that underlie ion channel regulation is a formidable task for neurobiologists. One aspect of this complex process that has received considerable attention recently is the modulation of channel activity by protein phosphorylation.

Many of the broad specificity kinases and phosphatases have been implicated in the regulation of a considerable number of channels, and several ion channel phosphorylation sites have been identified that could potentially have modulatory roles (Catterall, 1991; Levitan, 1999; Swope et al., 1999). However, elucidating the mechanisms by which these enzymes achieve specificity has been challenging. Certain kinases and phosphatases appear to be intimately associated with ion channels (Reinhart and Levitan, 1995; Yu et al., 1997). In the case of protein kinase A (PKA) and the type-1 phosphatase (PP-1), such specificity may be achieved by compartmentalization of the enzymes with their substrates. The focus of this minireview is to highlight advances on the targeting and functional coupling of PKA, and more recently PP-1, with ion channels through association with targeting subunits and anchoring proteins.

The AKAP Model

Subcellular localization of PKA occurs through association with A-kinase anchoring proteins (AKAPs). AKAPs are a functionally related family of proteins that are defined by their ability to associate with the PKA holoenzyme (Rubin, 1994; Colledge and Scott, 1999). Each anchoring protein contains at least two functional motifs. The conserved PKA binding domain forms an amphipathic helix, which slots into a hydrophobic pocket formed by residues in the extreme N terminus of the kinase's regulatory subunit dimer (RII). Secondly, each AKAP contains a unique targeting domain that directs the kinase/AKAP complex to a defined intracellular location. An additional feature of many AKAPs is their ability to form multivalent signaling complexes by associating with more than one enzyme (see Colledge and Scott, 1999, for references). Coordinate binding of specific combinations of enzymes could allow such complexes to respond to distinct second messenger–mediated signals.

Recently, reagents derived from the enzyme binding sites on AKAPs have been used to establish a role for these proteins in functional coupling to a variety of ion channels. Many of these studies were made possible by the development of an anchoring inhibitor peptide derived from the RII binding site of an AKAP (Ht31), which was shown to have a nanomolar binding affinity for the kinase and thus would antagonize RII/AKAP binding (Colledge and Scott, 1999). As this short peptide contained no intracellular targeting determinant, it was proposed to mediate a global uncoupling of PKA targeting when introduced into cells at a sufficiently high concentration.

Glutamate Receptors

Rosenmund et al. (1994) were the first to investigate a functional role for anchored PKA in neurons. Perfusion of the Ht31 anchoring inhibitor peptide into cultured rat hippocampal neurons promoted a time-dependent rundown of AMPA/kainate responsive currents, which had been shown previously to require PKA phosphorylation to maintain function (Rosenmund et al., 1994, and references therein). The PKA-specific inhibitor PKI mediated a similar rundown in current, while the Ht31 peptide effect could be overcome with perfusion of excess PKA catalytic subunit. Furthermore, a mutant Ht31 peptide, unable to antagonize RII/AKAP interactions, had no effect on the current. These experiments provided evidence of a role for kinase anchoring in PKA-mediated synaptic events; however, they did not specifically address which anchoring protein performed this role at synapses.

A more recent report from Greengard and colleagues suggests that phosphatase targeting may be equally important in the modulation of AMPA-type glutamate receptors (Yan et al., 1999). In an elegant series of biochemical and electrophysiological experiments, Yan et al. (1999) have demonstrated that the characteristic time-dependent rundown of AMPA receptors in neostriatal neurons is mediated by PP-1. AMPA currents were maintained by inhibiting the phosphatase with either okadaic acid or the activated inhibitor protein DARPP-32. Evidence for a more sophisticated level of regulation is suggested by data showing that the PP-1-targeting protein spinophilin, which is enriched in dendritic spines, may function to concentrate PP-1 close to the AMPA receptor. A peptide antagonist to the PP-1 binding site of spinophilin was able to maintain channel activity, presumably by uncoupling targeted PP-1, while having no direct effect on the catalytic activity of the phosphatase. It should be noted that this does not directly demonstrate that PP-1 is coupled to AMPA receptors via spinophilin in vivo. The PP-1 binding peptide, in a manner similar to the Ht31 peptide's disruption of anchored PKA, would mediate a widespread disruption of PP-1 from all targeting subunits. Nonetheless, these data support an interesting model for the dopaminergic modulation of glutamate channels in neostriatal neurons. Activation of D1-type dopamine receptors promotes PKA

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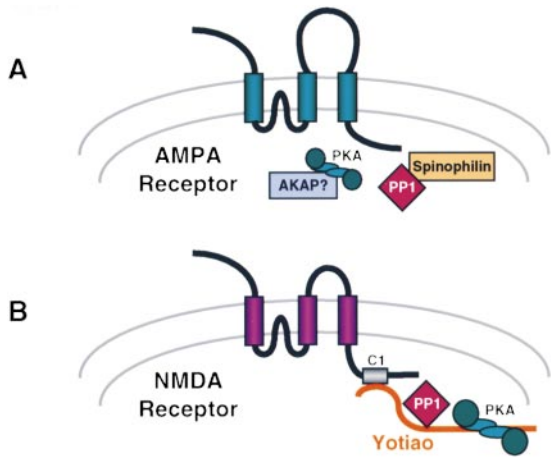

Figure 1. Signaling Enzymes and Anchoring Proteins at Glutamate Receptors

Schematic diagram depicting targeting of signaling enzymes to AMPA (A) and NMDA (B) glutamate receptors through anchoring proteins. In each case, a single receptor subunit is shown, four of which are proposed to assemble to form a functional channel.

phosphorylation of DARPP-32 that, in its phosphorylated form, can directly inhibit the anchored PP-1. Furthermore, AMPA channel activity is increased by direct PKA phosphorylation, which, when considered in light of the data from Rosenmund et al. (1994), could implicate a nearby pool of anchored kinase. However, the question of how PKA may be targeted to these channels remains open (Figure 1A).

More recently, it has been demonstrated that NMDA receptor subtypes also maintain their own anchored phosphatase/kinase signaling complex. Yotiao, a protein initially identified on the basis of its interaction with the C1 exon of the NR1 subunit of NMDA receptors, has been shown to target both PP-1 and PKA to the channel (Figure 1B; Westphal et al., 1999). Yotiao binds the kinase through a conventional PKA binding domain that is similar to other AKAPs, while a distinct region of the protein binds PP-1. Although yotiao is not the only AKAP that simultaneously binds a kinase and a phosphatase (Colledge and Scott, 1999), it is the first demonstration of an anchored signaling complex in which both enzymes coordinately regulate the activity of the same substrate. Enzymological analysis suggests that yotiao tethers PP-1 in an active state, favoring dephosphorylation under resting conditions. Support for this model comes from electrophysiological analysis of NMDA receptor currents. Tonic PP-1 activity attenuated receptor activity, as direct inhibition of the enzyme with okadaic acid led to an increase in glutamate-evoked currents. However, the same outcome was observed when a peptide derived from the phosphatase binding site of another PP-1-targeting protein, the muscle glycogen regulatory subunit (Gm), was included in the patch pipette. Since Gm has no inhibitory effect on PP-1 catalytic activity, this result parallels that observed with the spinophilin peptide and the AMPA receptor (Yan et al., 1999). Both studies demonstrate that not only is active PP-1 required, but that specific targeting of tonically active phosphatase may be an equally important event in the modulation of channel currents. Elevation of cAMP releases the anchored kinase to phosphorylate and upregulate channel activity. Since yotiao appears to be highly specific for C1 exon–containing NR1 subunits of NMDA receptors, it remains to be determined whether other NMDA receptors engage different AKAPs to perform a similar regulatory function. It is tempting to suggest that the requirement for locally anchored active phosphatase, observed with both NMDA and AMPA receptor subtypes (Westphal et al., 1999; Yan et al., 1999), may represent a common regulatory mechanism to maintain low-level activity for these channels in the absence of second messenger–mediated stimuli.

Ca^{2+} Channels

Voltage-dependent L-type Ca^{2+} channels are potentiated in response to sustained membrane depolarization or by trains of high-frequency depolarizations that mimic action potentials (Catterall, 1991). These ion channels were among the first shown to be dependent on phosphorylation, and biochemical studies demonstrated that kinase activity copurified with the channel. PKA was identified as the copurifying kinase and subsequently shown to be responsible for channel regulation through phosphorylation of the pore-forming $\alpha 1$ channel subunit (see Gray et al., 1997, for references).

PKA phosphorylation is essential for the prepulse potentiation and subsequent slow deactivation of skeletal muscle L-type Ca^{2+} channels. Since changes in the activity of these channels occurs rapidly on a millisecond timescale, it seemed surprising that PKA was capable of regulating this response since, upon cAMP activation, rapid phosphorylation of the channel would be hampered by diffusion limits on the catalytic subunit. Anchoring of PKA in close proximity to the channel could satisfy the need for rapid activation. In cultured myotubes, Catterall and colleagues demonstrated that anchoring inhibitor peptides prevented prepulse potentiation of L-type channels, suggesting that anchored PKA was indeed important for channel regulation (Johnson et al., 1994). Similar results were obtained using heterologously expressed skeletal muscle Ca^{2+} channel subunits in kidney cells (Johnson et al., 1997). The results of the latter study were notable in that PKA anchoring–dependent regulation was reconstituted without heterologous coexpression of an AKAP with the channel subunits. This suggests that an AKAP in the nonmuscle cell was capable of coupling with the expressed channel, although it should be noted that the same AKAP could be expressed in both cell types.

Cardiac L-type Ca^{2+} channels are also regulated by anchored PKA, although it has not been proven that prepulse potentiation of these channels is AKAP dependent. A membrane-targeted AKAP, AKAP79, was shown to reconstitute PKA regulation of the channel when expressed with cardiac Ca^{2+} channel subunits in HEK 293 cells (Gao et al., 1997). Since this anchoring protein is not expressed in cardiac tissue, it is possible that AKAP79, through its association with the plasma membrane, simply maintains PKA close to the channel in a heterologous expression system.

The possibility that AKAP79 is not coupled to L-type Ca^{2+} channels in vivo was supported by the detection of a low–molecular weight RII binding protein, named AKAP15, which copurified and coimmunoprecipitated

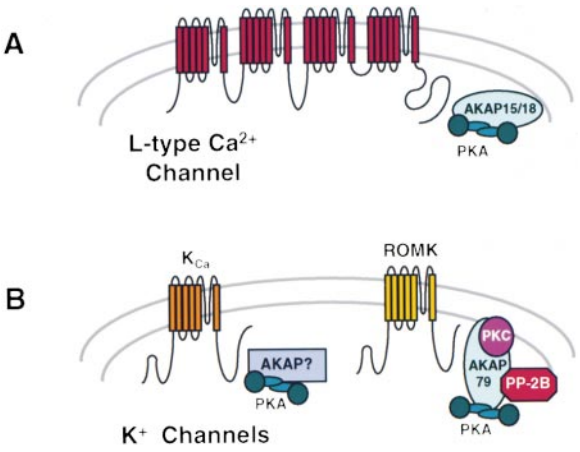

Figure 2. Signaling Enzymes and Anchoring Proteins at Ca^{2+} and K^+ Channels

(A) Targeting of PKA to the L-type Ca^{2+} channel through interaction with AKAP15/18. The schematic for the channel depicts the pore-forming α1 subunit, which is proposed to be the target for regulation by PKA phosphorylation.

(B) Targeting of signaling enzymes to K^+ channels. Single subunits for the large-conductance Ca^{2+}-activated K^+ channel (K_{Ca}) and the ROMK channel are shown. In each case, four such subunits assemble to form a complete channel.

with the Ca^{2+} channel from rabbit skeletal muscle (Gray et al., 1997). In parallel studies, a small 80-residue AKAP, named AKAP18, was cloned from a human brain cDNA expression library and shown to be targeted to the plasma membrane through N-terminal lipid modification. Myristoylation of the N-terminal glycine residue and dual palmitoylation of cysteines at positions 4 and 5 mediate AKAP18 targeting (Fraser et al., 1998). Subsequently, AKAP15 was cloned and found to be identical to AKAP18 (Gray et al., 1998a) (for the purposes of clarity, we will henceforth refer to this AKAP as AKAP15/18). Identification of AKAP15/18 mRNA in skeletal muscle, heart, brain, and pancreas is more consistent with the expression pattern of the L-type Ca^{2+} channel. Furthermore, detection of AKAP15/18 mRNA in kidney could account for the reconstitution of cAMP-dependent skeletal muscle channel activity in kidney-derived cells (Johnson et al., 1997). Since AKAP15/18 was capable of reconstituting cAMP-dependent channel activity when coexpressed with cardiac channel subunits (Fraser et al., 1998), and a peptide derived from the RII binding site of AKAP15/18 inhibited voltage-dependent potentiation of skeletal muscle channels (Gray et al., 1998a), it seems a more likely physiological partner for both skeletal and cardiac L-type Ca^{2+} channels than AKAP79 (Figure 2A). It has not yet been shown that AKAP15/18 directly interacts with the L-type Ca^{2+} channel, so it remains possible that coimmunoprecipitation of the two proteins involves an intermediate adaptor protein. It is worth noting that, more recently, AKAP15/18 has also been shown to coimmunoprecipitate with sodium channels purified from rat brain (Tibbs et al., 1998). Since these channels are inhibited by PKA phosphorylation, it is tempting to speculate that modulation of brain sodium channels will also depend on PKA anchoring.

Potassium Channels

Several protein kinases, including PKA, have been shown to regulate the activity of calcium-activated potassium channels (K_{Ca}) when added exogenously (Levitan, 1999). Wang and Kotlikoff (1996) identified PKA as an endogenous kinase responsible for the ATP-dependent augmentation of large conductance K_{Ca} channels in equine tracheal myocytes. Furthermore, a requirement for PKA anchoring was demonstrated when the Ht31 anchoring inhibitor peptide blocked ATP-dependent modulation of the channel (Figure 2B).

Another class of PKA-modulated potassium channel, the ROMK-like K^+ secretory channel (first cloned from a rat outer medulla kidney expression library), may also be modulated by a pool of anchored kinase. Ali et al. (1998) demonstrated that PKA-mediated activation of ROMK1, by either forskolin or the cAMP analog 8-bromo-cAMP, is dependent on coexpression of AKAP79 in *Xenopus* oocytes (Figure 2B). Once again, this illustrates that AKAP79 is able to promote phosphorylation of membrane-bound PKA substrates in a heterologous expression system. However, a ROMK-coupled AKAP of 100–120 kDa was identified by Ali et al. (1998), suggesting that AKAP79 is unlikely to be the physiological partner of the channel. A more likely candidate may be AKAP-KL, a family of alternatively spliced anchoring proteins enriched in kidney and lung that have been identified by Rubin and colleagues (Dong et al., 1998). Certain AKAP-KL isoforms migrate in the 100–120 kDa range and, like ROMK, are targeted to the apical membrane in kidney epithelia.

Conclusions

Given the central importance of ion channels in neuronal excitability, it is not surprising that several layers of regulation contribute to their tight control. While neurotransmitter binding or membrane depolarization are the primary stimuli for ligand-gated and voltage-gated ion channels, respectively, it is now clear that kinases and phosphatases play a prominent role in modulating the activity of these channels.

In addition, it appears that this fine tuning of channel activity is facilitated by the molecular organization of signaling enzymes with ion channels. This is where AKAPs and phosphatase targeting subunits play their part. AKAP15/18 and AKAP79 have been linked to several types of ion channels, although neither has yet been shown to bind directly to a specific channel subunit. As both anchoring proteins are targeted to submembrane sites through protein–lipid interactions, they maintain anchored pools of PKA close to ion channels. This might explain why these anchoring proteins appear to be functionally interchangeable in heterologous expression systems (Gao et al., 1997; Fraser et al., 1998).

In contrast, yotiao binds directly to C1 exon–containing NR1 subunits of NMDA receptors (Figure 1B; Westphal et al., 1999). Potentially, this may allow for greater precision in regulation, as the anchored pool of kinase and phosphatase are directed to a specific substrate. Such a sophisticated level of molecular organization may be particularly relevant for the precise control of synaptic transmission. Since NMDA receptors have been shown to be clustered at the postsynaptic density with other ion channels, physical association of the yotiao signaling complex with C1 exon–containing

receptors would enhance preferential modulation of these channels. However, an equally important property of yotiao may be to restrict access of PKA and PP-1 to other potential glutamate receptor substrates clustered nearby through interaction with PDZ domain–containing proteins such as PSD-95, Homer, and GRIP. We anticipate that the role of AKAPs in the modulation of ion channels will be clarified as our understanding of the molecular architecture supporting channel function improves.

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